Phytochemical screening and evaluation *in vitro of* antimicrobial activity of extracts of four Cameroonian medicinal plants on bacterial and fungal strains involved in skin lesion infections.

ABSTRACT

Background: Infections are recurrent in the presence of external skin lesions and significantly complicate the healing process. Plant extracts are used in the management of these complications. **Objective:** This study was aimed to evaluate the phytochemical composition and the *in vitro* antimicrobial (antibacterial and antifungal) activity of crude extracts of four Cameroonian medicinal plants. **Method**s**:** Primary and secondary metabolites were sought and quantified in the different aqueous and hydroethanolic extracts of these different plants through colorimetric tests and spectrophotometric assays. Determination of the Minimum Inhibitory Concentration (MIC) by Broth dilution methods were used to test the antimicrobial activity of different extracts of *Musa paradisiaca* , *Ficus exasperata* , *Dacryodes edulis* and *Citrullus lanatus* against five reference bacterial strains (*Shigella flexneri* NR 518, *Pseudemonas aeroginosa* NR 48982 ; *Staphylococcus aureus* NR 46003; *Klebsiela pneumonia* NR 41897, *Eschericha coli* ATCC 25922) and three fungal isolates (Candida *Albicans***;** *Candida parapsilosis***;** *Candida glabrata).* **Results:** Alkaloids, polyphenols (flavonoids, coumarins, total tannins), carbohydrates and total proteins were present in the different plant extracts with varying levels from one plant to another and from one extract to another. However, the *D. edulis* extracts had the highest levels overall. All the extracts tested inhibited the visible growth of one of the bacterial or fungal strains used, with MIC values between 1.565 and 25 mg/mL for bacterial strains and equal to 25 mg/mL for fungal isolates. The MIC values obtained with the reference molecules were between 0.015 and 0.0625 µg/mL and 0.0765 and 0.306 µg/mL respectively for Ciprofloxacin and Fluconazole. **Conclusion:** This study indicates clear evidence supporting the traditional use of *M. paradisiaca*, *F. exasperata*, *D. edulis* and *C. lanatus* in treating skin and wound infections related to bacterial and fungal.

Keywords: Antimicrobial activity**;** medicinal plants; secondary and primary metabolites; skin infection

INTRODUCTION

Skin lesions can be described as physical injuries that result in an opening or break in the skin. [1]. Physical, chemical, thermal, microbial, and immunological factors may be responsible for causing wounds in human and animals [2, 3]. Wound healing is a physiological process that restores the integrity of the skin after an injury, following several interdependent stages: haemostasis, inflammation, cell proliferation and remodelling. The existence of an infection may be the cause of the chronicity of a wound or a slowdown in the healing process. [4]**.** Thus, Skin infections and topical wounds require special attention as they make human and animal prone to bacterial, fungal, and viral contaminations, thereby making them further susceptible to other types of secondary complications [5]. The most common pathogens isolated from wounds are *Streptococcus* spp., *Staphylococcus aureus***,** *Escherichia coli***,** *Pseudomonas aeruginosa***,** *Proteus*

spp., *Klebsiella*, *Enterobacter*, *Enterococci*, *Bacteroides*, *Clostridium*, *Candida*, *Peptostreptococcus*, *Fusobacterium,* and *Aeromonas* [6]. Medicinal plants are effective in the treatment of infectious diseases and infections of various types of external wounds and have been used for these purposes in humans and different species of animals due to the side effects of modern medicine and lower price for herbal products [7,8]. Research reveals that several herbal treatments have marked activity in the management of infections of various types of external wounds and that this activity is ascribed to flavonoids, alkaloids, saponins, and phenolic compounds [7]. A preliminary study of the use of plants in the treatment of chronic and infected wounds carried out among traditional healers in the Bankim district (Adamaoua, Cameroon) identified five plants used for this purpose. Among these plants, *Musa paradisiaca*, *Ficus exasperata*, *Dacryodes edulis* and *Citrullus lanatus* were suggested as plant parts of therapeutic interest because of their use and their high number of citations compared with those of the other plants identified **[**9]. However, limited experimental evidence is available regarding the phytochemistry, the antimicrobial activity of those commonly used plants preparations. Hence, this study was aimed to investigate the qualitative and quantitative phytochemical compositions**,** the *in vitro* antibacterial activity of crude aqueous and hydroethanolic extracts of those selected medicinal plants against common bacterial and fungal involved in skin and wound infections.

METHODOLOGY

Study locations

The study took place at the laboratory for preclinical animal studies and pharmacotoxicology research of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I and at the Laboratory for Phytochemistry and Medicinal Plants Studies of the Sciences Faculty of the University of Yaoundé I, respectively for the phytochemical and microbiological study of the various extracts.

Plant material

The plant material consisted of drugs or parts of plants of interest used by certain traditional therapists in the Bankim Health District to treat chronic infected wounds. These were the leaves of *Musa paradisiaca* L., *Ficus exasperata* Vahl, *Dacryodes edulis* H.J. Lam and the pericarp of *Citrullus lanatus* Thunb. These plants were collected in December in Bankim and samples were identified at the National Herbarium of Cameroon in Yaoundé according to attestation N° 0264 / IRAD/ DG/ CRR- NK/ SSRG-HN/ SP-UR-PV/ 05/ 2021 of 23/08/2022.

Microorganisms

The microorganisms used included five (05) bacterial reference strains (*Shigella flexneri* NR 518, *Pseudemonas aeroginosa* NR 48982; *Staphylococcus aureus* NR 46003; *Klebsiela pneumonia* NR 41897, *Eschericha coli* ATCC 25922) and three (03) isolates of fungal strains (Candida *Albicans***;** *Candida parapsilosis***;** *Candida glabrata)* available at the Laboratory for Phytochemistry and Medicinal Plants Studies of the Sciences Faculty of the University of Yaoundé I.

Determination of the qualitative and quantitative phytochemical compositions of the various plant extracts

Preparation of extracts

For each plant, four extracts were prepared at 10% using two extraction solvents: water and ethanol at 50°. The aqueous extracts were obtained by maceration, decoction and infusion. The hydroethanol extract was obtained by maceration.

- Maceration procedure: The drugs from each plant, previously dried on a rack protected from light and at room temperature, were pulverized. 50g of powder from each plant was weighed using a METTLER DOLEDO® precision balance and placed in a 1000 mL Erlenmeyer flask. Next, 500 ml of the appropriate solvent was added (distilled water for the aqueous extract and water/ethanol 50/50 *v/v* for the hydro-ethanol extract). The mixture was homogenized as required and left to stand for 72 hours. The (macerated) product obtained was filtered using Whatman n°1 filter paper and the filtrate dried in a MEMMERT® model 200 ovens at a temperature of 40°C.

- Procedure for the decoction: The drugs from each plant, previously dried in a dark place at room temperature, were pulverized. 50g of powder from each plant was weighed using a precision balance and placed in a 1000mL Erlenmeyer flask. 500ml of distilled water was added. The mixture was homogenized and boiled in a water bath for 30 minutes. After cooling, the (decocted) product obtained was filtered using Whatman n°1 filter paper and the filtrate dried in an oven at a temperature of 40°C.

- Infusion procedure**:** The drug from each plant, previously dried in a dark place at room temperature, was pulverized. 50g of powder from each plant was weighed using a precision balance and placed in a 1000mL Erlenmeyer flask. 500 ml of distilled water, previously brought to the boil, was added. After homogenization and cooling, the (infused) product obtained was filtered using Whatman n°1 filter paper and the filtrate dried in an oven at a temperature of 40°C. Each dry extract obtained was weighed and stored in a clean, dry, sterile bottle. 2.4.2. Preparation of a stock solution of extract from each plant A stock solution of 1% extract of each plant was prepared. To this end, 0.5 g of dry extract was introduced into a beaker; 25 mL of distilled water was added and the mixture homogenized using a DRAGON LAB MSH-PRO+ magnetic stirrer until the dry extract was completely dissolved. The volume of the solution was then made up to 50 mL by adding distilled water to the mark.

Identification of groups of secondary metabolites present in plant extracts

The qualitative phytochemical analysis consisted of a series of colorimetric tests to characterise the groups of biological compounds contained in the crude extracts of the plants of interest according to the protocols of Ayoola et *al.* and Mehta & Patel [10, 11]. These main metabolites are alkaloids, polyphenols (flavonoids, tannins, coumarins), terpenoids, sterols, heterosides, saponosides, reducing sugars, lipids and proteins.

Quantitative determination of metabolite groups

Estimation of carbohydrates by the picric acid method

Procedure: In a glass tube, 100µL of the extract at a dose of 1000µg/ml was introduced. Then, 1000µL of 13% picric acid and 1000µL of 4% NaOH were added respectively. The tubes were boiled in a water bath for 10 minutes. In the white tube, the extract was replaced by distilled water. After the tubes were cooled, the optical density was read at 570nm against the blank and the extract concentration was obtained from the glucose calibration curve and expressed as glucose equivalent per mg of dry plant extract.

Estimation of total protein in extracts

It was carried out using the method of Lowry et *al*. [12].

Procedure: A volume of 1000 µl at a dose of 1000µg/ml of extract was added to 5mL tubes containing 2000 µl of Lowry D reagent. The mixture was mixed immediately (by inversion). The samples were then incubated for 10 min at room temperature. Next, 100µl of 1:10 diluted Folin Ciocalteu phenol reagent was added to each tube and vortexed immediately. After 30 min incubation at room temperature, the absorbance of each sample was read at 650 nm using a spectrophotometer. In the white tube, the extract was replaced by distilled water. The results obtained are expressed in µg of BSA per gram of dry matter from the calibration curve of a stock solution of bovine serum albumin (BSA) (100 µg / mL).

Estimation of total polyphenols in extracts

The amount of total phenolic content present in the samples was estimated using the FolinCiocalteu method described by Singleton et *al*. [13].

Procedure: A volume of 200 µl of each extract was introduced into glass haemolysis tubes. Subsequently, 1000 µl of 10-fold diluted Folin-Ciocalteu reagent and 800 µl of 7.5% sodium carbonate solution were added. The tubes were shaken and incubated for 120 min in the dark for the staining to develop. In the white tube, the extract was replaced with distilled water. The absorbance was read at 765 nm**.** A calibration curve was run in parallel under the same operating conditions using gallic acid at different concentrations (0 to 100 µg/ml). The measurement was repeated three times and the maximum error calculated. The quantity of phenolic compounds was expressed as the equivalent of mg of gallic acid per g of dry matter extract (mgGAE/gMS).

Estimation of total flavonoids in extracts

The quantity of flavonoids present in the extracts was determined by the aluminium method described by Zhishen et *al*. [14].

Procedure: In a 10ml test tube, 1000µl of extracts at a dose of 1000µg/ml, 150µl of 5% $NaNO_2$ and 150µl of 10% $AlCl_3 .6H_2 0$ were mixed. After 5 minutes' incubation, 1000µl of 4% NaOH was added. The solution was mixed and the absorbance was measured at 510 nm against the blank from which the extract had been replaced with distilled water. The standard curve for total flavonoids was performed using a standard solution of quercetin (0 to 100 µg/ml) following the same procedure as described above and expressed as milligrams of quercetin equivalents per g of dried fraction.

Estimation of total flavonoles

The method described by Miliauskas et *al*. [15] was used to determine the amount of total flavonols contained in the extracts.

Procedure: In a 10 ml test tube, 1000µl of extracts at a dose of 1000µg/ml, and 1000µl of $AlCl_3$ $6H_2O$ 2%, 600µl of sodium acetate 50g/l were introduced in turn. The volume was then made up to 3000µl with distilled water. The tubes were incubated at room temperature for 2.5h. The solution was mixed and the absorbance was measured against the blank not containing the extract at 440 nm. The standard curve for flavonols was determined using a standard quercetin solution (0 to 200 µg/l) using the same procedure as described above. Total flavonols were expressed as milligrams of quercetin equivalents per g of dried fraction.

Estimation of total tannins

The method described by Ali-Rachedi et *al*. [16] was used.

Procedure: In a 10 ml test tube, 1000µl of extract at a dose of 1000µg/ml, 200µl of Folin's reagent diluted to one tenth, 1000µl of Na $C0_{23}$ 35% were introduced. The solution was mixed and the absorbance was measured at 700 nm against the blank not containing the extract. The standard curve for total tannins was performed using a standard solution of tannic acid (0 to 500 µg/ml) following the same procedure as described above. Total tannins were expressed in milligrams of tannic acid equivalent per g of dried fraction.

Estimation of total alkaloids

The method described by Graham & Thomas [17] was used.

Procedure: Place 1000µl of the 1000µg/ml sample in a glass test tube, add 1000µl of 5% potassium dichromate and incubate the tubes for 5min at 30°C. Add 8000µl of concentrated sulphuric acid and vortex the tubes. Leave the tubes at room temperature for 20 minutes for the colour to develop, then read the optical density at 650nm against the blank or replace the sample with distilled water. The alkaloid concentration is obtained from the calibration curve and is expressed in milligram equivalents of Quinine hydrochlorhyde per gram of dried fraction.

Demonstration of the antibacterial and antifungal activities of the various extracts

The inhibition parameters of the various extracts were assessed by determining the Minimum Inhibitory Concentrations (MICs) using the liquid microdilution technique as described by the Clinical and Laboratory Standards Institute (CLSI), protocol M07-A9 for bacteria [18] and Clinical and Laboratory Standards Institute (CLSI)protocol M27-A3) for yeasts [19].

Preparation of stock solutions of extracts and reference antimicrobials

Stock extract solutions were prepared at 100 mg/mL by dissolving 100mg of each extract in 1ml of 10% DMSO. Ciprofloxacin and fluconazole were prepared under the same conditions, at 1mg/mL by dissolving 1ml of powder in acidified sterile distilled water (5% hydrochloric acid) and were used as a positive control during the tests.

Preparation of culture media

For antibacterial activity, the agar culture media, Mueller Hinton (MHA) required for strain subculturing and Mueller Hinton broth (MHB) for microdillution were prepared according to the manufacturer's recommendations. To this end, 24 g of medium powder was dissolved in 1 L of distilled water; the resulting mixture was then autoclaved at 121 ± 1°C for 15 minutes. The MHA medium was poured into Petri dishes before cooling.

For antifungal activity, Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were prepared according to the manufacturer's recommendations. To this end, 30 g of medium powder was dissolved in 1 L of distilled water to prepare the SDA and SDB media respectively. The resulting mixtures were autoclaved at 121 ± 1°C for 15 min. The SBA medium was then poured into Petri dishes before cooling.

Preparation of bacterial and fungal inocula

Bacterial inocula were prepared according to the 0.5 Mc Farland standard. To this end, a few bacterial colonies from a pure culture of 18-24 h on Mueller Hinton agar were homogenized in 10mL of physiological water to obtain a turbidity equivalent to the 0.5 tube of the Mc Farland standard range ($1.5x10^8$ CFU/mL). The suspension was then diluted with 1/100th MHB to obtain an inoculum with a concentration of 10^6 CFU/mL [20]. This procedure was the same for each of the bacterial strains tested.

Fungal inocula were prepared according to the 0.5 Mc Farland standard. For this purpose, a stock suspension was prepared at a turbidity of 0.5 Mc Farland (corresponding to an approximate concentration of $2.5x10^6$ cells/mL) from young cultures grown for 48h on Sabouraud Dextrose Agar (SDA), collected with a platinum loop and introduced into a tube containing 5 ml of sterile physiological water; then diluted to 10^4 CFU/ml for the tests. [21]. This procedure was the same for each of the four fungal strains selected.

Determination of Minimum Inhibitory Concentrations (MICs) for bacteria and yeasts

The liquid microdilution technique as described by the Clinical and Laboratory Standards Institute (CLSI), protocol M07-A9 for bacteria [20] and CLSI, protocol M27-A3 for yeasts [21] was used.

Procedure: The tests were carried out in duplicate in sterile 96-well microplates. Fifty µL of culture medium (MHB for bacteria and SDB for yeasts) was introduced into each well of the plate. Next, 50 µL of a sterile solution of each extract sample concentrated to 100 mg/mL was taken and introduced into the first corresponding wells, followed by a series of 5 dilutions of geometric reason of order 2. Finally, 50 µL of a bacterial suspension with a load of $1x10^6$ CFU/mL (antibacterial tests) and a fungal suspension with a load of $2x10^4$ CFU/mL (antifungal tests) were distributed to the test and negative control wells. The concentrations of the extracts, ciprofloxacin and fluconazole in the wells ranged from 25 mg/mL to 0.781 mg/mL for the extracts and from 1.9 µg/mL to 0.015 µg/mL for the reference substances. The final inoculum load in each well was $5x10^5$ CFU/mL for the antibacterial tests and 10^4 cells/mL for the antifungal tests.

The sterility control consisted solely of the culture medium. The positive control consisted of the culture medium, inoculum and ciprofloxacin for antibacterial tests and fluconazole for antifungal tests. The microplates were coated and incubated at 37°C for 24 hours for the antibacterial tests and 48 hours for the antifungal tests. At the end of the incubation period, 10µL of a newly prepared resazurin solution (0.15 mg/mL) was added to all wells and the plates were again incubated under the same conditions for 30 minutes. The lowest concentration at which no change in coloration from blue to pink was observed, corresponding to no visible growth of the bacteria or yeast, was taken as the MIC for the extract concerned.

RESULTS

Phytochemical characterization

Qualitative phytochemical composition of the various plant extracts

Table 1 presents the results of the qualitative phytochemistry of the families of primary and secondary metabolites explored in the various extracts of *D. edulis, M. paradisiaca, C. lanatus* and *F. exasperate*.

Alkaloids, polyphenols (flavonoids, coumarins, total tannins), carbohydrates, total proteins and vitamin C are present in all extracts. However, the absence of other families of compounds, namely oxalates, resins, anthraquinones and chalcones, was observed in all the different extracts.

Certains groups of compounds such as carotenoids, anthocyanins and anthocyanins were present only in the various extracts of *M. paradisiaca.* The Keller Killiani test, revealing the presence of cardiac glycosides, was positive only with the various extracts from the pericarp of *C. lanatus*. There was an absence of phlobotanins in the various *D. edulis* extracts and an absence of quinones and betacyans in the various *M. paradisiaca* leaf extracts. Saponosides were present in the ethanolic extract of *F. exasperata* and in the various extracts of *M. paradisiaca* and *D. edulis*.

Table 1: Qualitative phytochemical composition of various plant extracts

Plants	***Dacryodes edulis***				***Citrullus lanatus***				***Musa paradisiaca***				***Ficus exaperata***			
Metabolites	**a**	**b**	**c**	**d**	**a**	**b**	**c**	**d**	**a**	**b**	**C**	**d**	**a**	**b**	**c**	**d**
Lipids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vitamin C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carotenoids	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Cholesterol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyphenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucilage	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponosides	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxalates	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
Terpenoids and Steroids	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Phlobotanins	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Quinones	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
Betacyans	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
Chalcones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonols / Flavones	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-

Legend: a = Ethanolic extract; b= Aqueous extract by maceration; c= Extract by infusion; d= Extract by decoction; + (Present) and - (Absent)

Quantitative phytochemical composition of the various plant extracts

Table 2 shows the carbohydrate, protein, total polyphenol, flavonoid, flavonol, total tannin and alkaloid contents of the various extracts from the leaves of *M. paradisiaca*, *D. edulis*, *F. exasperata* and the pericarp of *C. lanatus*.

Among the *M. paradisiaca* extracts, the aqueous extract obtained by infusion had the highest levels of the metabolites tested. In ascending order of content in this extract: carbohydrates (20.38 ± 0.11 E mg Glucose/gMS), total tannins (114.07 ± 11.70 E mg Tannic acid/gMS), proteins (162.82 ± 2.22 E µg BSA/gMS), flavonoids (178.29 ± 18.65 E mg quercetin/gMS) and polyphenols (246,53 ± 2.54 E mg Gallic Acid/gMS) were present at lower levels than flavonoles (942.38 ± 34.78 E mg Quercetin/gMS) and alkaloids (1449.44 ± 13.47 E mg Quinine hydrochloride /gMS).

Among the *D. edulis extracts*, the aqueous extract by maceration had the highest levels of carbohydrates (15.26 ± 0.43 E mg Glucose/gMS), total tannins (149.16 ± 6.59 E mg Tannic acid/gMS) and flavonoids (772.26 ± 16.06 E mg quercetin/gMS); the hydroethanol extract had the highest levels of flavonoids (229.18 ± 0.78 E mg quercetin/gMS) and total protein (464.36 ± 12.34 E µg BSA/gMS); the aqueous extracts obtained by decoction and infusion had the highest levels of polyphenols (346.00 ± 5.23 E mg Gallic Acid/gMS) and alkaloids (1665.00 ± 90.18 E mg Quinine hydrochloride /gMS) respectively. In ascending order of content in these extracts: carbohydrates, total tannins, flavonoids, polyphenols and proteins were present at lower levels than flavonols and alkaloids.

The aqueous extract obtained by maceration of the leaves of *F. exasperata*, had the lowest metabolite content; the hydroethanol extract had the highest polyphenol content (232.67 ± 0.83 E mg Gallic Acid/gMS); the aqueous extract obtained by decoction had the highest levels of total protein (124.87 ± 10.33 E µg BSA/gMS) and flavanols (665.95 ± 22,86 E mg Quercetin/gMS) and the extract obtained by infusion had the highest contents of carbohydrates (16.06 ± 0.73 E mg Glucose/gMS), flavonoids (91.53 ± 5.10 E mg Quercetin/gMS) and total tannins (172.47 ± 0.66 E mg Tannic acid/gMS)). In ascending order of content in these extracts: carbohydrates, flavonoids, proteins, total tannins and polyphenols were present at lower levels than flavonols and alkaloids.

Among the *C. lanatus* extracts, the aqueous extract obtained by maceration had the highest total tannin content (99.04 ± 1.19 E mg Tannic acid/gMS); the hydroethanolic extract had the highest total protein content (238.72 ± 14.48 E µg BSA/gMS) and alkaloid content (1569.44 ± 26.94 E mg Quinine hydrochloride/gMS). the aqueous extract obtained by decoction had the highest carbohydrate content (10.31 ± 0.05 E mg Glucose/gMS) and the extract obtained by infusion had the highest levels of polyphenols (250.67 ± 2.66 E mg Gallic acid/gMS), flavonoids (64.57 ± 0.45 E mg Quercetin/gMS) and flavonoles (675.12 ± 31.66 E mg Quercetin/gMS). In ascending order of metabolite content in these extracts, carbohydrates, flavonoids, total tannins, proteins and polyphenols were present at lower levels than flavonols and alkaloids.

Table 2: Content of certain groups of metabolites contained in the various plant extracts

Plant	Type of extract	Carbohydrates (E mg G/gMS)	Protein (E µg BSA/gMS)	Polyphenols (E mg GAE/gMS)	Flavonoids (E mg Quer/gMS)	Flavonoles (E mg Quer/gMS)	Total tannins (E mg Tannic acid/gMS)	Alkaloids (E mg Quinine/gMS)
Musa paradisiaca	Aqueous macerate	19,76 ± 0,38	135,13 ± 1,18	163,47 ± 1,62	136,82 ± 4,08	700,83 ± 61,45	76,31 ± 1,31	1235,00 ± 82,12
	Ethanolic	16,64 ± 0,28	135,90 ± 2,70	244,00 ± 7,03	115,06 ± 10,03	803,93 ± 46,47	108,85 ± 4,09	1431,6 7 ± 52,39
	Decocted	17,52 ± 0,88	143,59 ± 2,22	219,87 ± 4,77	120,06 ± 2,06	776,90 ± 82,15	92,55 ± 2,96	1286,11 ± 57,19
	Infused	20,38 ± 0,11	162,82 ± 2,22	246,53 ± 2,54	178,29 ± 18,65	942,38 ± 34,78	114,07 ± 11,70	1449,44 ± 13,47
Dacryodes edulis	Aqueous macerate	15,26 ± 0,43	427,18 ± 5,40	338,53 ± 8,39	223,88 ± 4,04	772,26 ± 16,06	149,16 ± 6,59	1531,67 ± 27,28
	Ethanolic	12,81 ± 0,35	464,36 ± 12,34	337,33 ± 4,28	229,18 ± 0,78	675,95 ± 75,39	145,58 ± 4,41	1599,44 ± 34,69
	Decocted	11,41 ± 0,05	454,87 ± 40,60	346,00 ± 5,23	183,10 ± 9,10	652,62 ± 67,10	127,46 ± 7,68	1571,67 ± 21,86
	Infused	13,12 ± 1,37	416,64 ± 2,46	339,73 ± 17,64	226,43 ± 10,45	692,50 ± 57,39	142,49 ± 4,10	1665,00 ± 90,18
Ficus exaspera	Aqueous	14,86 ± 0,09	122,31 ± 7,81	191,60 ± 13,76	75,16 ± 1,89	518,21 ± 36,28	161,61 ± 1,33	1275,00 ± 55,08

ta	macerate							
	Ethanolic	14,10 ± 0,51	119,15 ± 14,88	232,67 ± 0,83	72,61 ± 1,22	590,00 ± 71,81	166,61 ± 1,51	1480,56 ± 34,05
	Decocted	15,69 ± 0,33	124,87 ± 10,33	208,00 ± 11,22	81,53 ± 1,79	665,95 ± 22,86	166,22 ± 2,73	1273,89 ± 20,37
	Infused	16,06 ± 0,73	122,56 ± 3,95	226,93 ± 3,59	91,53 ± 5,10	570,00 ± 90,85	172,47 ± 0,66	1193,89 ± 145,08
	Aqueous macerate	10,03 ± 0,14	162,82 ± 17,48	226,13 ± 20,92	63,59 ± 3,11	672,62 ± 45,97	99,04 ± 1,19	1382,78 ± 48,80
Citrullus lanatus	Ethanolic	9,91 ± 0,66	238,72 ± 14,48	241,47 ± 10,28	62,41 ± 5,23	670,00 ± 60,00	96,31 ± 1,42	1569,44 ± 26,94
	Decocted	10,31 ± 0,05	188,46 ± 15,90	243,47 ± 3,20	52,61 ± 1,48	580,36 ± 130,27	91,82 ± 2,32	1470,56 ± 78,20
	Infused	9,76 ± 0,26	178,72 ± 27,10	250,67 ± 2,66	64,57 ± 0,45	675,12 ± 31,66	97,28 ± 2,82	1502,78 ± 27,15

Values are expressed in terms of mean ± standard deviation, (n = 3). Comparisons between groups were made using the variance test (ANOVA

Minimum Inhibitory Concentrations of the various extracts in relation to the strains tested

Table 3 shows the Minimum Inhibitory Concentration (MIC) values for the extracts tested against the reference bacterial strains and fungal isolates used.

All the extracts tested inhibited the visible growth of one of the bacterial or fungal strains used, with MIC values between 1.565 and 25 mg/mL for the bacterial strains used and equal to 25 mg/mL for the fungal isolates, with the exception of the aqueous extracts by maceration and infusion of *Ficus exasperata* and infusion of *Citrullus lanatus*. The MIC values obtained with the reference molecules were lower than those obtained with the various extracts; these values ranged between 0.015 and 0.0625 µg/mL and 0.0765 and 0.306 µg/mL respectively for Ciprofloxacin and Fluconazole The sensitivity to the extracts varied according to the type of microbial strain used: *P. aeroginosa* was sensitive to 50% of the extracts tested, *K. pneumonia* to 68.75%, *E. coli* to 43.75%, *S. flexneri* to 43.75%, *S. aureus* to 37.5%, *C. albicans* to 43.75%, C. *parapsilosis* to 37.5% and *C. glabrata* to 18.75%.

The MIC values obtained with the various *C. lanatus* pericarp extracts ranged from 6.25 to 25 mg/mL. The aqueous extract by decoction inhibited the growth of all the bacterial strains tested. The reference strain *K. pneumonia* proved to be the most sensitive to the various *C. lanatus* extracts. The aqueous extract by maceration of *M. paradisiaca* inhibited the growth of all the bacterial strains tested with MIC values between 6.25 and 25 mg/mL. Only the aqueous extract obtained by decoction of *D. edulis* leaves and the hydroethanolic extract of *F. exasperata* inhibited the growth of all the bacterial strains tested.

With regard to fungal isolates, only aqueous extracts by decoction of *F. exasperata* leaves, *D. edulis* and *C. lanatus* pericarp inhibited the visible growth of *C. albicans***.** The various extracts of *M. paradisiaca* inhibited the growth of all the fungal isolates tested

Table 3: Minimum inhibitory concentrations (MICs) of different plant extracts against bacterial and fungal strains

PLANT	TYPE OF EXTRACT	MIC (mg/mL)							
		PA	KP	EC	SF	SA	CA	CP	CG
Musa paradisiaca	Aqueous macerate	25	6,25	25	>25	25	25	25	25
	Ethanolic	>25	3,125	>25	>25	>25	25	25	25
	Decocted	>25	>25	25	>25	>25	25	25	25
	Infused	25	6,25	>25	12,5	25	25	25	25
Dacryodes edulis	Aqueous macerate	6,25	3,125	1,562	1,562	25	>25	25	>25
	Ethanolic	1,562	3,125	>25	3,125	>25	>25	>25	>25
	Decocted	3,125	3,125	6,25	1,562	25	25	>25	>25
	Infused	12,5	6,25	>25	6,25	>25	>25	>25	>25
Ficus exasperata	Aqueous macerate	>25	>25	>25	>25	>25	>25	>25	>25
	Ethanolic	12,5	6,25	12,5	6,25	25	>25	>25	>25
	Decocted	>25	>25	12,5	>25	>25	25	>25	>25
	Infused	>25	>25	>25	>25	>25	>25	>25	>25
Citrullus lanatus	Aqueous macerate	>25	25	>25	>25	>25	>25	>25	>25
	Ethanolic	>25	6,25	>25	>25	>25	>25	>25	>25
	Decocted	12,5	6,25	25	12,5	25	25	>25	>25
	Infused	>25	>25	>25	>25	>25	>25	>25	>25
Contrôle positif	Ciprofloxacine (µg/mL)	0,031	0,015	0,031	0,0625	0,015	NA	NA	NA
	Fluconazole (µg/mL)	NA	NA	NA	NA	NA	0,0765	0,153	0,306

SF : *Shigella flexneri* NR 518, PA : *Pseudemonas aeroginosa* NR 48982 ; SA : *Staphylococcus aureus* NR 46003 ; KP : *Klebsiela pneumonia* NR 41897, EC : *Eschericha coli* ATCC 25922 ;CA : *Candida Albicans* ; CP : *Candida parapsilosis* ; CG : *Candida glabrata* ; >25 : not active ; NA : not applicable.

DISCUSSION

The results of the qualitative phytochemistry of certain primary and secondary metabolites explored in the various *D. edulis* leaf extracts revealed the presence of alkaloids, polyphenols (flavonoids, coumarins, total tannins, gallic and catechic tannins, betacyans, flavonols and flavones, quinones, cardiac glycosides, anthraquinones), carbohydrates, total proteins and vitamin C in all types of extracts. Terpenoids and steroids, phlobotanins, anthocyanins, saponosides, chalcones, oxalates, phlobotanins, carotenoids and total lipids were absent in all aqueous extracts. Similar results have been observed with extracts of various organs of this plant by many authors; alkaloids, phenoles, flavonoids, triterpenoids, tannins, saponins, anthocyanins and anthraquinones were present in aqueous extracts of *D. edulis* leaves; only steroids were not detected. [22,23]. Sap from the stem presented numerous bioactive compounds such as: saponins, alkaloids, tannins, flavonoids and other phenolic compounds [24]. However, the saponosides present in previous studies were absent from the *D. edulis* leaf extracts in the present study.

Extracts of *M. paradisiaca* leaves showed that, with the exception of lipids, all the other primary metabolites we were looking for were present in the various extracts. Polyphenols (tannins, flavonoids and coumarins) and alkaloids were present in all extracts. Oxalates, resins, cardiac glycosides and terpenoids were not. With the exception of the terpenoids absent in the present study, similar results have been obtained by numerous authors who have reported the presence of tannins, saponosides, terpenoids and steroids, phenols, flavonoids and alkaloids in extracts of various solvents and organs of the plant (*M. paradisiaca*): leaves, stem, fruit pulp and bract [25,26,27-32] .

The various extracts of the pericarp of *C. lanatus* showed the presence in all extracts of alkaloids, polyphenols (flavonoids, coumarins, total tannins, gallic and catechic tannins), quinones, terpenoids and steroids, betacyans, phlobotanins, carbohydrates, total proteins, vitamin C and carotenoids. Mucilages are present in the hydroethanoic and aqueous extracts obtained by infusion and absent in the aqueous extracts obtained by aqueous maceration and the aqueous extract obtained by decoction. Anthocyanins, saponosides, cardiac glycosides, oxalates, phlobotanins and total lipids were absent in all extracts. With the exception of the saponins and oxalates absent in the present study, similar results have been obtained with extracts of various plant solvents and drugs by many authors. Indeed, *C. lanatus* seed and pericarp extracts showed the presence of alkaloids, phenols, flavonoids, tannins, saponins , steroids and terpenoids [33-36].

The results of the qualitative phytochemical screening of the different *F. exasperata* leaf extracts revealed the presence of numerous primary and secondary metabolites, including: polyphenols (flavonoids, tannins, phlobotanins, coumarins, quinones), mucilages, alkaloids, carbohydrates, proteins and vitamin C in the different extracts. Other metabolites such as oxalates, resins, anthocyanins, cardiac glycosides, betacyans, chalcones, anthocyanins and anthraquinones were absent in the various extracts. The results of numerous studies on the qualitative phytochemical composition of extracts of leaves and bark of the plant revealed the presence of the same metabolites present in the extracts of the present study [37-40]. However, cardiac glycosides and oxalates, absent in the present study, are present in the work of previous authors.

The contents of carbohydrates, total proteins, total polyphenols, flavonoids, flavonols, total tannins and alkaloids were determined in the various extracts. Summaries of the contents of certain metabolites present in these extracts of plants of interest revealed that alkaloids followed by flavonols were the most preponderant.

The metabolite content of the various *D. edulis* leaf extracts varied from one extract to another. In ascending order, carbohydrates, total tannins, flavonoids, polyphenols and proteins were present, with lower levels than flavonols and alkaloids. Similar results were obtained by Chinelo et *al*. [41] who found that alkaloid and flavonoid levels were highest compared to other metabolites in the leaves, fruit pulp and seeds of the same plant. However, the proportions obtained in the present study are different (lower) from those reported by Wego Kamgaing et *al*. [22]in Cameroon, who worked on the aqueous extract obtained by maceration of the leaves, and those of Ogboru et *al*. [42], in Nigeria and Ano-Aka et *al*. [43] in Côte d'Ivoire, who worked respectively on the rind and pulp of the fruit of the same plant. These results suggest that the harvesting site and the part of the plant used also have an impact on the quantity of secondary metabolites in the plant.

Of the *M. paradisiaca* leaf extracts, the aqueous extract obtained by infusion had the highest metabolite content. This finding is consistent with the fact that the extraction technique and the nature of the solvent have an impact on metabolite content [44,45] In fact, water is a polar solvent that is suitable for extracting polar compounds such as phenolic compounds. In addition, increasing the heat increases the solubility of the chemical constituents and therefore the extractive properties of the solvent. In ascending order of metabolite content in these extracts, carbohydrates, total tannins, proteins, flavonoids and polyphenols were present at lower levels than flavonols and alkaloids. These results differ from those obtained by Falowo et *al*. in Nigeria, who found that alkaloids and flavonoids were present in lower concentrations than saponins and tannins [27].

Among the *C. lanatus* extracts, the aqueous extract obtained by maceration had the highest content of total tannins; the hydroethanolic extract had the highest content of total proteins and alkaloids; the aqueous extract obtained by decoction had the highest carbohydrate content and the extract obtained by infusion had the highest content of polyphenols, flavonoids and flavonoles. In ascending order of content in these extracts: carbohydrates, flavonoids, total tannins, proteins and polyphenols were present at lower levels than flavonoids and alkaloids. Among the *F. exasperata* extracts, the aqueous extract obtained by maceration had the lowest levels of metabolites; the hydroethanol extract had the highest levels of polyphenols; the aqueous extract obtained by decoction had the highest levels of total proteins and flavanols and the extract obtained by infusion had the highest levels of carbohydrates, flavonoids and total tannins. In ascending order of content in these extracts: carbohydrates, flavonoids, proteins, total tannins and polyphenols were present at lower levels than flavonols and alkaloids.

Recognizing the role and importance of phytotherapy, the World Health Organisation (WHO) has, over the years, put in place a number of strategic plans and adopted measures to combat infectious diseases; this is the case with the use of plants which, through their metabolisms, produce a wide range of bio-active metabolites, such as alkaloids, flavonoids, saponins and

tannins, which are capable of inhibiting growth and interfering negatively with the metabolism of microorganisms; thus justifying antibacterial and antifungal activities [46,47].

In the present study, the antibacterial and antifungal activities of different extracts from the leaves of *M. paradisiaca, D. edulis*, *F. exasperata and* the pericarp of *C. lanatus* were tested with the aim of identifying extracts potentially active against certain bacterial and fungal strains infecting wounds, such as *P. aeroginosa, K. pneumonia, E. coli, S. flexneri, S. aureus* and *C.* albicans [48]. In the present study, with the exception of aqueous extracts by maceration and infusion of *Ficus exasperata* and infusion of *Citrullus lanatus*, all the extracts tested inhibited the visible growth of at least one bacterial or fungal strain among those used, with MIC values between 1.565 and 25 mg/mL for bacterial strains and equal to 25 mg/mL for fungal isolates. Sensitivity to the extracts varied according to the type of microbial strain used: *P. aeroginosa* was sensitive to 50% of the extracts tested, *K. pneumonia* to 68.75%, *E. coli* to 43.75%, *S. flexneri* to 43.75%, *S. aureus* to 37.5%, *C. albicans* to 43.75%, C. *parapsilosis* to 37.5% and *C. glabrata* to 18.75%. The presence and high metabolite content of these extracts are thought to be responsible for this microbiological activity.

The aqueous extract by maceration of *M. paradisiaca* leaves inhibited the growth of all the bacterial strains tested, with MIC values ranging from 6.25 to 25 mg/mL. Many researchers have reported similar results; Asuquo & Udobi evaluated the antibacterial potential of the ethanolic extract of *M. paradisiaca* leaf and its aqueous fraction against numerous germs (*S. aureus, B. subtilis, P. aeruginosa, V. cholerae* and *S. dysenteriae*) and showed that the aqueous fraction had better antibacterial activity than the ethanolic extract with minimum inhibitory concentration values for the aqueous fraction of between 3.125 and 25 mg/ml [49]. Other researchers, such as Alisi et *al*. [50] demonstrated the antimicrobial activity of the aqueous extract of unripe fruit peels and leaves of *M. paradisiaca* against Staphylococcus and Pseudomonas species. However, Ahmad et Beg [51] reported that the alcoholic extract of *M. paradisiaca* stem showed no activity against *S. aureus, S. paratyphi, S. dysenteriae, E. coli, B. subtilis* and *C. albicans*. Karadi et *al*. [52] reported significant antimicrobial activity of methanolic extract of *M. paradisiaca* fruit epicarp against bacteria *E. coli, S. aureus, B. subtilis, P. aeruginosa* and fungi *C. albicans, C. tropicalis, A. niger*.

Only the aqueous extract obtained by decoction of *D. edulis* leaves and the hydroethanolic extract of *F. exasperata* inhibited the growth of all the bacterial strains tested. The presence of polyphenols in these extracts at slightly higher levels than the other extracts (346 ± 5.23 and 232.67 ± 0.83 respectively for the aqueous extract by decoction of *D. edulis* and hydro ethanolic extract of *F. exasperata*) would justify this observation. Indeed, phenolic acids (cinnamic and benzoic) which are phenolic compounds are known to have antibacterial, antiparasitic antifungal, antioxidant properties among others [27].

The MIC values obtained with the different *C. lanatus* pericarp extracts ranged from 6.25 to 25 mg/mL. The aqueous extract by decoction inhibited the growth of all the bacterial strains tested, as well as *C albicans*. Numerous studies have demonstrated the antimicrobial activity of *C. lanatus* seeds on bacterial and fungal strains, including *K. pneumoniae*, P. *aeruginosa, V. cholerae, P. mirabilis, S. dysentriae, S. aureus, E. coli, E. faecalis, S. typhi, B. subtilis* and *C.*

albicans. [53,554]. Other studies, such as those by Siti Suhaila et *al*. [35] have reported the antimicrobial activity of organic extracts (methanolic and hexanolic) from the epicarp of *C. lanatus* against *Staphylococcus epidermidis* and *Trichophyton mentagrophytes.* The antimicrobial activity of these extracts can be explained by the presence of metabolites with antibacterial and antifungal properties, such as polyphenols, which act by weakening the membrane of microorganisms. [35,53,554]

CONCLUSION

This study focused on the phytochemical composition and antimicrobial activity against bacterial and fungal strains of various extracts obtained from the leaves of *Musa paradisiaca*, *Ficus exasperata*, *Dacryodes edulis* and the pericarp of *Citrullus lanatus*. Numerous metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, carbohydrates and proteins were identified in the different extracts at concentrations justifying the inhibition of the growth of many of the strains tested. All the plant species evaluated in this study are currently used traditionally for the treatment of skin and wound infections. The positive findings from this study provide a scientific basis for the traditional use of *M. paradisiaca*, *F. exasperata, D. edulis* et *C. lanatus* have a promising antibacterial activity against tested microorganism. Finally, the results of this study clearly elucidate the antibacterial and antifungal potential of these plants and provide an evidence to support their use in folk medicine.

Ethical Approval: Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. clearance number: 0065/UYI/FMSB/VDRC/DAASR/CAD

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